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POLYPHENOLS IN SOIL PROFILE DEVELOPMENT

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Search for a common denominator in such diverse soil phenomena as the accumulation of surface organic debris, the formation of humic acid, and the weathering of inorganic substances, points to the rôle of polyphenols. These vary, in amount and kind in leaves, according to species of plant, site characters and season. In addition, polyphenols may be biosynthesized and may also be formed during soil degradation.

Since beech and oak can grow on extremes of acid and of base-rich sites, comparison has been made possible of leaf polyphenols in these at the two extremes, especially in relation to tanning ability. Differences in this are relevant to the stability of superficial humus, but cannot explain that of humic acid and of the mineral soil in the absence of clear evidence for stabilized protein.

In humic acid the theory of a stable polyphenol-amino acid complex polymer is preferred, and comparisons are drawn between the hydrolysate of a model catechol-glycine oxidation polymer, and that of humic acid.

Polyphenols can reduce ferric iron, and then form a non-ionic complex capable of moving freely in soil solution without ionic exchange. This explains the presence of altered polyphenol in the B horizon of a podsol; from this is deduced a theory of the movement of iron compounds in leached soils in general.

A scheme is outlined for the rôle of polyphenols in soil.

The development of a soil profile involves a complex variety of processes. The tendency in all early investigations on soils has been to examine each process in isolation, but a stage has been reached where the processes must be studied as a whole, and the separate effects integrated.

Three processes commonly studied in isolation are:—

(a) The formation and nature of surface humus, in particular that formed during the decay of leaves.

(b) The nature of the organic matter in the soil proper, especially that part known as humic acid.

(c) The degradation of soil minerals, and processes involved in movement of iron, aluminium, and similar inorganic materials.

By surface or superficial humus we mean the surface cover of organic matter resulting from decomposition of litter, largely above the surface of the inorganic soil. This commonly occurs in woodland soils, in heaths, in acid grassland, and, in an extreme form, in peat.

Humic acid we define as humic acid of soil, i.e. the complex mixture of organic polyanions of soil, soluble in alkali but insoluble in acids (Davies & Coulson, 1959). General consideration of these processes will show that they are all influenced by common features. They are all dependent on the nutrient status, drainage, and on both atmospheric and soil climate of the site in question. These in turn modify the influence of the plant species contributing the organic substances, the whole processes in time approaching a state of equilibrium.

Handley (1954) in his Technical Communication 'Mull and Mor', both from his own observations and the work of others, attributed differences between Mull and Mor superficial humus to differences in stability of the tanned proteins of leaves;

those leaves growing on Mor sites being more effectively stabilized against microbiological attack than those on Mull sites. There are various indices of the effective stability of Mor superficial humus, e.g. unavailability of the nitrogen, continued existence of cellulose, and of chlorophyll. These are not absolute indices, for the microbiological rate may be influenced by the inorganic nutrient status and physical condition of the soil.

The stability of organic matter, within the soil profile as opposed to resting upon it, has been given several explanations but common to many have been the stability of lignin-protein and lignin-ammonia complexes (cf. Waksman 1936; Mattson and Koutler-Anderson 1943; see also Bremner 1956). Recent work by Morrison (1958) has confirmed lignin-like substances in humic acid, more particularly in that of peat, but there is no convincing evidence for peptide bonds within this organic matter (Swaby 1958*ab;* 1959*b* and private communication), not even in the soil ligno-protein isolated according to Tinsley and Zin (Coulson private communication). After examining Australian soils exhaustively over a period of two years, Swaby came to the conclusion that their humic acid contained neither lignin nor protein. The concept of stable tanned proteins suited to superficial humus cannot be extended to the humic acid within the soil.

In recent years Flaig (1955) and Swaby (1958*ab;* 1959*b* and private communication) have revived the old ideas on quinone-amino acid polymers, while Kononova (1958) has emphasized the part played by polyphenols produced by micro-organisms.

In connection with the third process (c), Bloomfield (1957, 1958) has shown that unidentified polyphenolic substances in plant leaves can be held responsible for the movement of iron.

In our search for a common denominator in the processes governing the nature and extent of organic and inorganic development of a soil profile, we are led to the belief that polyphenols are the unifying factor. The amount and kind of polyphenols in the growing leaves of the plants suited to each site is particularly relevant to superficial humus and to the movement of some inorganic substances within the profile. This belief is consistent with many of the observations of the investigators cited, and is firmly grounded in our own work.

Superficial humus.

The degree of stability of litter and superficial humus against microbiological degradation varies from one tree species to another, the stability being least for those growing in base- or nutrient-rich sites. Beech, which will grow on soils of a wide range of nutrient status, can produce Mull at one extreme of nutrition and Mor at the other. Oak is somewhat similar (Fig. 1). We have examined fallen dead leaves, litter and humus of beech and oak from characteristic Mull and Mor sites. While those of Mor sites contain polyphenols extractable by a tannin stripping method (methanol reflux) those of Mull sites do not. Both materials after hydrolysis and reduction released large amounts of polyphenol. This suggests that though polyphenols are present in both in some form or other, only in Mor sites has the polyphenol been considerably concerned with tanning of protein. We have also examined both beech and oak leaves just prior to senescence, and find that cold methanol-extractable polyphenols from the leaves of Mull sites contained a lesser quantity of gelatin-precipitating tannins than those of Mor sites. In general tree species associated with base-deficient sites (both cool-temperature and tropical) are richer in these extractable tannins, especially in leucoanthocyanins. We have shown that gelatin-tannin precipitates formed at low pH (2.65) are more stable to protease *in vitro* than those formed at other pH. Both phenomena could explain the superior stability of organic nitrogen compounds in superficial humus of Mor sites in terms of tanned proteins, but cannot be accepted as explaining the stability of humic acid.

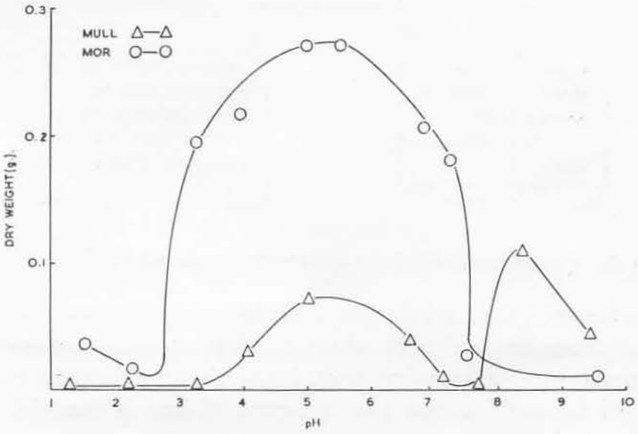

FIGURE 1. The tannin-gelatine precipitates formed in tanning baths of pH within the range 1–10. The tannins were isolated from oak leaves growing on trees situated on mull and mor sites.

Humic Acid.

Any theory concerning the nature of nitrogen compounds in humic acid must accommodate the lack of evidence for protein nitrogen especially in mineral soils. It is not sufficient to assume that liberation of amino-acids on acid hydrolysis is proof of protein nitrogen, since the amino-acids could have been bound in some other manner. Nor is it right to assume that evidence gathered from a study of peat—relatively little altered from fresh plant substance—suffices to explain the behaviour of humus of inorganic soils; the humic acids from these representing two extreme forms of development (Coulson, Davies & Khan 1959).

Examination of the reduced hydrolysate of a catechol-glycine oxidation polymer showed that not only is the glycine recoverable but some of the original catechol and its oxidation polymers also. When humic acid of peat and of a clay loam were so hydrolysed and reduced, similar fragments of polyphenol and their polymers were recoverable along with the usual amino-acids (Coulson *et al.* 1959). This is consistent with the Flaig-Swaby concept of quinone-amino acid polymerization as the basis of humic acid common to both peaty and inorganic soils. This does not exclude the probability that in soil humic acids rich in lignin-like substances (e.g. peats) the lignin may also serve to protect amino-acids.

We would suggest that humic acid can form in the soil as shown in Fig. 2.

It would seem that most of the ideas put forward are based on facts connected with materials of a phenolic nature or capable of acting as a source of phenolic substances.

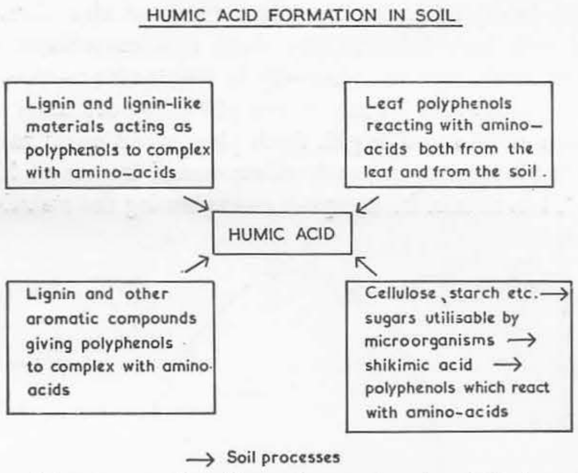
HUMIC ACID FORMATION IN SOIL
Lignin and lignin-like materials acting as polyphenols to complex with amino-acids
Leaf polyphenols reacting with amino-acids both from the leaf and from the soil.
HUMIC ACID
Lignin and other aromatic compounds giving polyphenols to complex with amino acids
Cellulose, starch etc. → sugars utilisable by microorganisms → shikimic acid → polyphenols which react with amino-acids
→ Soil processes

FIGURE 2. Scheme suggesting the *main* ways humic acid is formed in the soil.

Processes involving inorganic substances, especially the movement of iron.

Since in all freely-drained soils where rainfall exceeds evaporation, hydrated iron oxide occurs to degrees varying from base-rich brown earths to acid podzols, an explanation of the mobilization and transport of iron is essential for an understanding of soil genesis.

We have found *D*- and *epi*-catechin present in growing green leaves of many

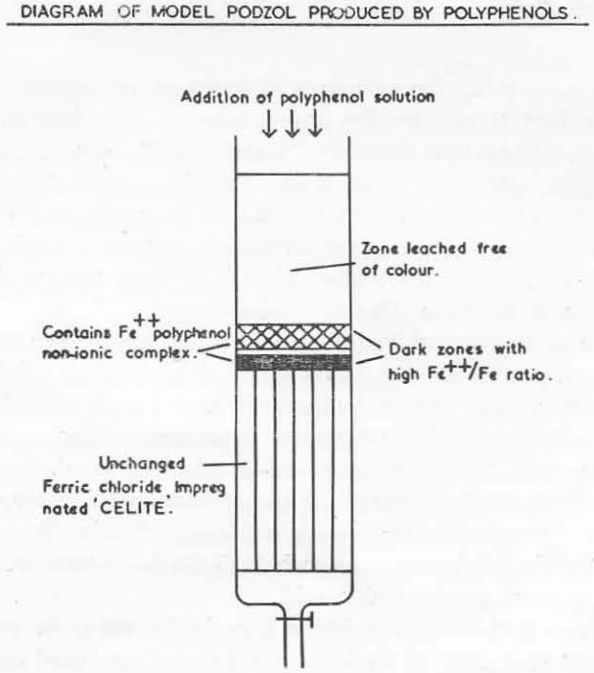
DIAGRAM OF MODEL PODZOL PRODUCED BY POLYPHENOLS.
Addition of polyphenol solution
Zone leached free of colour.
Contains Fe++ polyphenol non-ionic complex.
Dark zones with high Fe++/Fe ratio.
Unchanged Ferric chloride impregnated 'CELITE'.

FIGURE 3. Diagrammatic representation of model podzol produced when polyphenol solutions (D-catechin or *epi*-catechin or leaf isolates) pass through columns of 'Celite 535' which has been impregnated with ferric chloride solution.

trees (Coulson, Davies & Lewis 1960*a*). By *in vitro* experiments we have shown that *D*- and *epi*-catechin are capable of reducing ferric iron and forming stable complexes with the ferrous iron especially in mildly acid conditions (pH 4-5) (cf. Fig. 3). These complexes are water soluble and, being non-ionic, are not subject to exchange phenomena. They will, in consequence, move freely in the profile (Coulson, Davies & Lewis 1960*b*).

The amount of polyphenol in growing leaves varies inversely with the nutrient status of the soil (Stitt, Hyland & McKee 1946; Jenkinson 1958; Swaby 1958; Henson private communication; Coulson *et al.* 1960*a*). Its fate on reaching the soil will be determined by the soil reaction—the more acid the soil, the more stable the polyphenol. We therefore find polyphenol activity to be lowest on base-rich sites. Contrary to the high level of easily available polyphenols in the growing leaves is the low level in litter and superficial humus, even on Mor sites. Our experience has shown that the polyphenol responsible for movement of iron in soils is likely to be that washed from the growing leaves into the soil, and not that from litter or humus. We have systematically collected rain washings from leaves of various tree species, and concentrated them by low-temperature evaporation and lead acetate precipitation. Initial results over a period of two months in a rather exceptional season (May, June, 1959) are in agreement with our hypotheses (see Table 1).

Table 1

Polyphenols in rain dripping from leaves

Soil: Stony medium loam. Penrhyn series
(Llandegai : May and June, 1959)

Batch No.	Amount of polyphenol (various units)	Spruce	Austrian Pine	Beech	Sycamore	Japanese Larch
1 (9.5. 1959 - 13.5.1959)	Weight of polyphenol (g/sq.yd.)	0.016	0.012	0.024	0.024	0.016
	Reducing power (g Fe^{++} produced/g polyphenol)	0.011	0.006	0.005	0.003	0.005
	Fe^{++} complexing potential (g Fe^{++}/sq.yd. X 10^{-4})	1.8	0.7	1.2	0.7	0.8
2 (13.5.1959 - 21.5.1959)	Weight of polyphenol (g/sq.yd.)	0.056	0.014	0.148	0.064	0.164
	Reducing power (g Fe^{++} produced/g polyphenol)	0.025	0.021	0.012	0.015	0.008
	Fe^{++} complexing potential (g Fe^{++}/sq.yd. X 10^{-4})	14.0	3.0	17.8	9.6	13.2
3 (21.5.1959 - 5.6.1959)	Weight of polyphenol (g/sq.yd.)	0.10	0.188	0.08	0.08	0.128
	Reducing power (g Fe^{++} produced/g polyphenol)	0.02	0.06	0.03	0.02	0.046
	Fe^{++} complexing potential (g Fe^{++}/sq.yd. X 10^{-4})	20.0	113.0	24.0	16.0	58.8
4 (5.6.1959 - 18.6.1959)	Weight of polyphenol (g/sq.yd.)	0.046	0.105	0.102	0.495	0.127
	Reducing power (g Fe^{++} produced/g polyphenol)	0.046	0.019	0.036	0.017	0.013
	Fe^{++} complexing potential (g Fe^{++}/sq.yd. X 10^{-4}	21.2	20.0	36.7	82.4	16.5
	Total Fe^{++} complexing potential (g Fe^{++}/sq.yd. X 10^{-4})	57.0	136.7	79.7	110.5	89.3

In the brown earth the fate of the iron complex is not easy to unravel because of the mixing of the soil by earth worms. In the podzol, where earth worms are absent, the transported iron is deposited in a clearly defined horizon—the B zone. We have examined samples of this horizon and have found that the organic matter freed from its cation complexes by the acetyl acetone-method of Martin and Reeves (1953) (about 50% of the total organic matter) yielded substantial amounts of polyphenol on acid hydrolysis, evidence not only for the presence of polyphenol but for its association with the iron.

There can be no doubt that any satisfactory explanation of the movement of iron must be based on activity of polyphenol. We visualise the rôle of polyphenols derived from leaves and by microbial activity in the scheme shown in Figure 4.

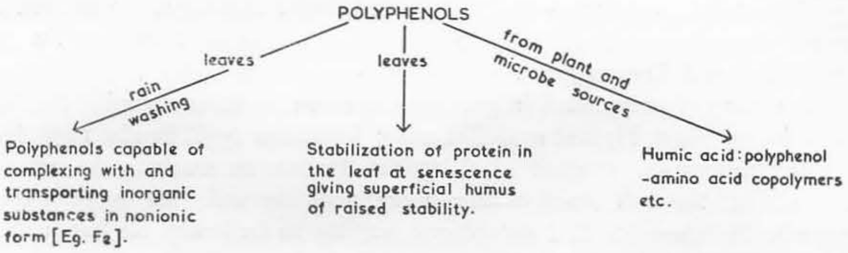
POLYPHENOLS
leaves
leaves
from plant and microbe sources
rain washing
Polyphenols capable of complexing with and transporting inorganic substances in nonionic form [Eg. Fe].
Stabilization of protein in the leaf at senescence giving superficial humus of raised stability.
Humic acid: polyphenol -amino acid copolymers etc.

FIGURE 4. General scheme outlining the role of polyphenols in soil profile development.

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